REMARKS

Claims 1, 3-7, 9, 10, 21-28, 30-33, 35 and 36 presently appear in this case. No claims have been allowed. The official action of September 13, 2004, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

Briefly, the present invention relates to a fused chimeric protein that is a linear genetically-engineered molecule of amino acid residues connected by peptide bonds. The chimeric protein is produced by fusing at the level of cDNA, DNA encoding at least one cell-targeting moiety, and DNA encoding at least one cell-killing moiety. The cell-targeting moiety is Met-GnRH or a Met-GnRH analog that specifically binds to GnRH binding sites on Caco2 adenocarcinoma cells. The cell-killing moiety is preferably a bacterial toxin, such as *Pseudomonas* exotoxin (PE). The invention further relates to pharmaceutical compositions and methods of treating various conditions by administering the chimeric protein of the present invention. Among the conditions that may be treated are adenocarcinomas and hepatocarcinomas, benign uterine leiomyoma, extrauterine endometriomas, benign hyperplasia of prostate or breast, and pituitary tumor adenoma.

The interview between Examiner Helms and the undersigned attorney on December 9, 2004, is hereby gratefully acknowledged. In the course of the interview the issues were discussed, and the examiner confirmed that, while he concedes

that applicant has shown that the properties of the claimed products are unexpectedly different from the properties of the prior art product, it was the examiner's position that since it would be obvious to make the change to the prior art product in order to obtain the product of the present invention, whatever properties are obtained would be inherent, and thus would not make the new product patentable. Applicant's attorney explained that a showing of unexpected results would rebut a *prima facie* case of obviousness, and that which is inherent is not necessarily known, and that obviousness cannot be predicated on what is unknown. Applicant's attorney promised to cite the appropriate case law in response to the official action, and the examiner stated that he would carefully reconsider the issue upon consideration of such case law.

The examiner has objected to claim 36 as depending from a cancelled claim. Claim 36, as well as claim 35, have now been amended so as to depend from claim 30 rather than cancelled claim 29. Accordingly, this objection has now been obviated.

Claims 1, 3-7, 21, 23, 28, 30-33, and 35 and 36 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Nett, and further in view of Chaudhary (1989) and Chaudhary (1987), and as evidenced by the specification. The examiner states that the declaration of Dr. Lorberbaum-Galski has been carefully considered but is not deemed to be

persuasive. The examiner states that the references disclose that it is routine in the art to add an ATG codon to the 5' of the nucleic acid to express the fusion protein in *E. coli*, and therefore there would be no difference between the claimed fusion proteins and that of the prior art. As to the fact that the claimed proteins recognize and target different receptors, and can target and kill adenocarcinoma cells, the examiner states that molecules that would result from the combination of Nett and Chaudhary would be the same as those claimed, and as such would obviously target the adenocarcinoma cell binding sites. The examiner states that the product claimed and the product produced by the combination of the prior art would result in targeting the binding sites on adenocarcinoma cells, because the products are the same. This rejection is respectfully traversed.

As discussed at the above-mentioned interview, it is effectively the examiner's position that, while he concedes that the evidence of record shows unexpected properties in the product of the present invention, the invention as a whole would still be obvious, because such properties would be inherent when one adds a a Met to the GnRH of Nett for the reasons disclosed by Chaudhary. However, this logic fails on many bases. First of all, Chaudhary does not teach any motivation to add a Met to the GnRH of Nett. Indeed, Chaudhary is silent as to whether or not the initial Met is even present. The examiner assumes it is present, as this is

a common side effect of bacterial production. It does not appear that Chaudhary ever even checked to see whether or not an N-terminal Met was present. Accordingly, this is not a case where desirable characteristics are disclosed for a combined product and applicant has merely discovered additional characteristics. Indeed, it is understood in the art that the presence of such a Met is usually undesirable. Attached hereto are some abstracts to this effect that have been found in a brief search, including:

Adinolfi et al, "Full antitumor action of recombinant seminal ribonuclease depends on the removal of its N-terminal methionine", Biochem Biophys Res Commun. 213:525-532 (1995)

Liao et al, "Removal of N-terminal methionine from recombinant proteins by engineered E. coli methionine aminopeptidase", Protein Sci. 13:1802-10 (2004) ["The removal of N-terminal translation initiator Met by aminopeptidase(MetAP) is often crucial for the function and stability of proteins."]

Chaudhuri et al, "Effect of the extra N-terminal methionine residue on the stability and folding of recombinant alpha-lactalbumin expressed in Escherichia coli", J Mol Biol. 285:1179-1194 (1999) ["Although the overall structures of the authentic and recombinant proteins are the same, the extra methionine residue at the N-terminus of the recombinant protein remarkably affects the native-state stability and the electrical properties."]

Mauguilevsky et al, "Production of authentic human proapolipoprotein A-I in Escherichia coli: strategies for the removal of the amino-terminal methionine", J Biotechnol. 27:159-172 (1993).

It is well established that the presence of an unexpected property is evidence of non-obviousness, even with

respect to a chemical compound. In this regard, the examiner's attention is invited to MPEP 716.02(a)III, which states:

> **III. PRESENCE OF AN UNEXPECTED PROPERTY IS EVIDENCE OF NONOBVIOUSNESS**
>
> Presence of a property not possessed by the prior art is evidence of nonobviousness. *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963) (rejection of claims to compound structurally similar to the prior art compound was reversed because claimed compound unexpectedly possessed anti-inflammatory properties not possessed by the prior art compound); *Ex parte Thumm*, 132 USPQ 66 (Bd. App. 1961) (Appellant showed that the claimed range of ethylene diamine was effective for the purpose of producing " 'regenerated cellulose consisting substantially entirely of skin' " whereas the prior art warned "this compound has 'practically no effect.' "). The submission of evidence that a new product possesses unexpected properties does not necessarily require a conclusion that the claimed invention is nonobvious. *In re Payne*, 606 F.2d 303, 203 USPQ 245 (CCPA 1979). See the discussion of latent properties and additional advantages in MPEP § 2145.

The examiner's attention is also invited to MPEP 2144.09, and particularly in the section titled "*Prima Facie* Case Rebuttable by Evidence of Superior or Unexpected Results", which states:

> A *prima facie* case of obviousness based on structural similarity is rebuttable by proof that the claimed compounds possess unexpectedly advantageous or superior properties. *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963) (Affidavit evidence

> which showed that claimed triethylated compounds possessed anti-inflammatory activity whereas prior art trimethylated compounds did not was sufficient to overcome obviousness rejection based on the homologous relationship between the prior art and claimed compounds.); *In re Wiechert*, 370 F.2d 927, 152 USPQ 247 (CCPA 1967) (a 7-fold improvement of activity over the prior art held sufficient to rebut *prima facie* obviousness based on close structural similarity).
>
> However, a claimed compound may be obvious because it was suggested by, or structurally similar to, a prior art compound even though a particular benefit of the claimed compound asserted by patentee is not expressly disclosed in the prior art. It is the differences in fact in their respective properties which are determinative of nonobviousness. If the prior art compound does in fact possess a particular benefit, even though the benefit is not recognized in the prior art, applicant's recognition of the benefit is not in itself sufficient to distinguish the claimed compound from the prior art. *In re Dillon*, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991).
>
> See MPEP § 716.02 - § 716.02(g) for a discussion of evidence alleging unexpectedly advantageous or superior results.

Note that the case of *In re Dillon* is not applicable here because the declaration shows that there are actual differences between the prior art compound and the claimed compound, not just a discovery of differences that had not been recognized by the prior art.

As to the examiner's comments about the inherency of the product obtained by the proposed combination, the

examiner's attention is invited to *In re Henderson*, 146 USPQ 372, 375 (CCPA 1965), which states, with respect to a very similar fact situation:

> We are not impressed by the board's view, here urged by the solicitor, that because the ether additives would "inherently function" to produce the decreased sensitivity, the claimed blends are therefore obvious. Indeed, can it not be said that all functions of a substance are "inherent"? The fact remains that appellant has discovered that when certain ethers are blended with certain gasolines, the sensitivity of the resulting gasoline composition is less than the sensitivity of either the ether or the gasoline alone. The mere notion that such discovery would "flow naturally" from what appellant did does not mean that the discovery could be predicted from what the art had done. As we said in *In re Papesch, 50 CCPA 1084, 315 F.2d 381, 137 USPQ 43:* "From the standpoint of patent law, a compound and all of its properties are inseparable; they are one and the same thing."

See also *In re Spormann*, 150 USPQ 449, 452 (CCPA 1966), quoted with approval in *In re Shetty*, 195 USPQ 753 (CCPA 1977:

> As we pointed out in *In re Adams*, 53 CCPA 996, 356 F.2d 998, 148 USPQ 742, the inherency of an advantage and its obviousness are entirely different questions. That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown.

Applicant concedes that the examiner has established a *prima facie* case of obviousness. However, the totally surprising and unexpected results that are obtained insofar as

the properties of the Met-GnRH-toxin are concerned rebuts this *prima facie* case of obviousness. The unexpected results cannot be ignored. They are not possessed by the prior art compound, and they certainly could not have been predicted from what the art had done. Accordingly, the compounds as well as the methods of the present invention are unobvious and patentable over the combination of references cited by the examiner. Reconsideration and withdrawal of this rejection is therefore respectfully urged.

It is submitted that all of the claims now present in the case clearly define over the references of record. Reconsideration and allowance are therefore earnestly solicited.

Respectfully submitted,

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1: Biochem Biophys Res Commun. 1995 Aug 15;213(2):525-32. Related Articles, Links

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Full antitumor action of recombinant seminal ribonuclease depends on the removal of its N-terminal methionine.

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Bovine seminal RNase (BS-RNase) is a dimeric member of the pancreatic-like ribonuclease superfamily, with antitumor activity. We report here that recombinant Met(-1) BS-RNase is a less potent cytotoxic factor, while structurally and catalytically indistinguishable from BS-RNase isolated from natural sources. Mature recombinant BS-RNase instead displays full antitumor action. This suggests that the conformation of the N-terminal region of BS-RNase is among the structural determinants of its antitumor action, in addition to its catalytic activity and its quaternary structure.

PMID: 7646508 [PubMed - indexed for MEDLINE]

1: Protein Sci. 2004 Jul;13(7):1802-10.

Removal of N-terminal methionine from recombinant proteins by engineered E. coli methionine aminopeptidase.

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The removal of N-terminal translation initiator Met by methionine aminopeptidase (MetAP) is often crucial for the function and stability of proteins. On the basis of crystal structure and sequence alignment of MetAPs, we have engineered Escherichia coli MetAP by the mutation of three residues, Y168G, M206T, Q233G, in the substrate-binding pocket. Our engineered MetAPs are able to remove the Met from bulky or acidic penultimate residues, such as Met, His, Asp, Asn, Glu, Gln, Leu, Ile, Tyr, and Trp, as well as from small residues. The penultimate residue, the second residue after Met, was further removed if the antepenultimate residue, the third residue after Met, was small. By the coexpression of engineered MetAP in E. coli through the same or a separate vector, we have successfully produced recombinant proteins possessing an innate N terminus, such as onconase, an antitumor ribonuclease from the frog Rana pipiens. The N-terminal pyroglutamate of recombinant onconase is critical for its structural integrity, catalytic activity, and cyto-toxicity. On the basis of N-terminal sequence information in the protein database, 85%-90% of recombinant proteins should be produced in authentic form by our engineered MetAPs.

PMID: 15215523 [PubMed - in process]

1: J Mol Biol. 1999 Jan 22;285(3):1179-94. Related Articles, Links

Erratum in:
- J Mol Biol. 2004 Feb;336(3):825.

Effect of the extra n-terminal methionine residue on the stability and folding of recombinant alpha-lactalbumin expressed in Escherichia coli.

Chaudhuri TK, Horii K, Yoda T, Arai M, Nagata S, Terada TP, Uchiyama H, Ikura T, Tsumoto K, Kataoka H, Matsushima M, Kuwajima K, Kumagai I.

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The structure, stability, and unfolding-refolding kinetics of Escherichia coli-expressed recombinant goat alpha-lactalbumin were studied by circular dichroism spectroscopy, X-ray crystallography, and stopped-flow measurements, and the results were compared with those of the authentic protein prepared from goat milk. The electric properties of the two proteins were also studied by gel electrophoresis and ion-exchange chromatography. Although the overall structures of the authentic and recombinant proteins are the same, the extra methionine residue at the N terminus of the recombinant protein remarkably affects the native-state stability and the electric properties. The native state of the recombinant protein was 3.5 kcal/mol less stable than the authentic protein, and the recombinant protein was more negatively charged than the authentic one. The recombinant protein unfolded 5.7 times faster than the authentic one, although there were no significant differences in the refolding rates of the two proteins. The destabilization of the recombinant protein can be fully interpreted in terms of the increased unfolding rate of the protein, indicating that the N-terminal region remains unorganized in the transition state of refolding, and hence is not involved in the folding initiation site of the protein. A comparison of the X-ray structures of recombinant alpha-lactalbumin determined here with that of the authentic protein shows that the structural differences between the proteins are confined to the N-terminal region. Theoretical considerations for the differences in the conformational and solvation free energies between the proteins show that the destabilization of the recombinant protein is primarily due to excess conformational entropy of the N-terminal methionine residue in the unfolded state, and also due to less exposure of hydrophobic surface on unfolding. The results suggest that when the N-terminal region of a protein has a rigid structure,

expression of the protein by E. coli, which adds the extra methionine residue, destabilizes the native state through a conformational entropy effect. It also shows that differences in the electrostatic interactions of the N-terminal amino group with the side-chain atoms of Thr38, Asp37, and Asp83 bring about a difference in the pKa value of the N-terminal amino group between the proteins, resulting in a greater negative net charge of the recombinant protein at neutral pH. Copyright 1999 Academic Press.

PMID: 9887272 [PubMed - indexed for MEDLINE]

☐ 1: J Biotechnol. 1993 Jan;27(2):159-72. Related Articles, Links

Production of authentic human proapolipoprotein A-I in Escherichia coli: strategies for the removal of the amino-terminal methionine.

Moguilevsky N, Varsalona F, Guillaume JP, Gilles P, Bollen A, Roobol K.

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Several methods were compared with respect to the production of authentic, N-terminal methionine-free proapolipoprotein A-I in engineered Escherichia coli bacteria. A first approach consisted of treating the purified methionylated recombinant protein with an amino-peptidase, purified from Aeromonas proteolytica. A second series of strategies was based on the construction of proapo A-I encoding cassettes carrying built-in recognition sites suitable for specific in vitro cleavage of the products with kallikrein and enterokinase, respectively. Along the same line, a fusion between ubiquitin and proapo A-I was produced in E. coli with the prospect to achieve post-purification cleavage with yeast ubiquitin hydrolase. Finally, proapo A-I was fused to the signal peptide of the bacterial outer membrane protein, OmpA, aiming at an in situ conversion to authentic proapo A-I during secretion to the bacterial periplasm. The data showed that, out of these five systems, the OmpA signal peptide system and, to a lesser extent, the one involving the fusion to ubiquitin were the most efficient in yielding authentic proapo A-I from engineered Escherichia coli.

PMID: 7763464 [PubMed - indexed for MEDLINE]